



Heat and pressure treatments effects on peanut allergenicity

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ABSTRACT

Peanut allergy is recognized as one of the most severe food allergies. The aim of this study was to investigate the changes in IgE binding capacity of peanut proteins produced by thermal-processing methods, including autoclaving. Immunoreactivity to raw and thermally processed peanut extracts was evaluated by IgE immunoblot and skin prick test in patients with clinical allergy to peanut. Roasted peanut and autoclaved roasted peanut were selected for IgE ELISA experiments with individual sera, immunoblot experiments with antibodies against peanut allergens (Ara h 1, Ara h 2 and Ara h 3), digestion experiments, and circular dichroism spectroscopy. *In vitro* and *in vivo* experiments showed IgE immunoreactivity of roasted peanut proteins decreased significantly at extreme conditions of autoclaving. Circular dichroism experiments showed unfolding of proteins in autoclave treated samples, which makes them more susceptible to digestion. Autoclaving at 2.56 atm, for 30 min, produces a significant decrease of IgE-binding capacity of peanut allergens.

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1. Introduction

Peanut allergy is one of the most severe food allergies due to its life-threatening nature and persistency (Sicherer & Sampson, 2007). Peanut allergy prevalence seems to have increased in the western world during the past decades. An estimate of the prevalence of peanut allergy in US children was 1.4% in 2008 compared with 0.8% in 2002 and 0.4% in 1997 in a self-reported population survey (Sicherer, Muñoz-Furlong, Godbold, & Sampson, 2010).

Foods, including peanuts, are subjected to different processing methods to improve their quality, preservation, safety, suitability for specific product applications, etc. (Sathe & Sharma, 2009). The types of modifications that food proteins may undergo during processing includes protein unfolding and aggregation and chemical modification (Mills, Sancho, Rigby, Jenkins, & Mackie, 2009). These modifications could alter food allergenicity, i.e. increasing or decreasing IgE reactivity (Cabanillas-Martín, Crespo, Burbano, & Rodríguez, 2010; Cuadrado et al., 2009). Therefore, understanding food processing seems to be important in food allergenicity.

It has been recognized that roasted peanuts are more allergenic than raw peanuts (Beyer et al., 2001; Chung & Champagne, 1999, 2001; Kopper et al., 2005; Maleki, Chung, Champagne, & Raufman, 2000; Maleki et al., 2003). Roasted peanut extracts bind IgE from

patients with peanut allergy at approximately 90-fold higher levels than raw peanuts, and the protein modifications induced by Maillard reaction contribute to the observed effect (Chung & Champagne, 1999, 2001; Maleki et al., 2000). Previous studies have demonstrated that autoclaving at 2.56 atmospheres (atm) for 30 min (min) and “instantaneous controlled pressure drop (DIC)” at 6 bar (5.9 atm) during 3 min reduced IgE-binding capacity of lupine allergens, whereas it was only slightly affected by boiling, microwave and extrusion-cooking, demonstrating the high thermal resistance of the major lupine allergens (Alvarez-Alvarez et al., 2005; Guillamón et al., 2008).

Autoclaving at 2.56 atm, for 30 min, produced a relevant decrease in the IgE-binding capacity of lentil and chickpea allergens. However, several immunoreactive proteins still remained in these legumes upon harsh autoclaving (Cuadrado et al., 2009).

We sought to investigate the changes in IgE binding capacity of peanut proteins produced by thermal-processing methods, including autoclaving.

2. Materials and methods

2.1. Patients and sera

Serum samples from 54 patients sensitized to peanut were used in this study. Nineteen of them (Nos. 1–19) had a clinical history of peanut allergy, confirmed on the basis of either a convincing history of severe systemic anaphylaxis after peanut ingestion or a

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positive double-blind, placebo-controlled food challenge (DBPCFC) with peanut, a positive skin prick test response and a specific IgE level to peanut, greater than 0.35 kU/l (median = 2.79 kU/L; range, 0.47–100 kU/L), quantified with the CAP-FEIA System (Phadia, Uppsala, Sweden). The other 35 patients (Nos. 20–54) were sensitized to peanut, having a specific IgE level to peanut, greater than 0.35 kU/l (median = 5.44 kU/l; range, 0.36–100 kU/L). A serum pool from 4 out of 19 patients with actual peanut allergy (Nos. 5, 8, 9 and 11) and individual sera from all patients were used in IgE immunodetection assays. A serum pool from three patients with specific IgE to *Anisakis* spp. was used as a negative control.

After informed consent, seven additional patients with confirmed clinical allergy to peanut underwent skin testing with raw and thermally processed peanut extracts. Two healthy subjects were tested with the same protein extracts as a control group. The study was approved by the Ethics Committee of the Hospital Universitario 12 de Octubre of Madrid (Permission No. 0312150129).

2.2. Plant material, heat treatments and protein extracts

Raw, fried and roasted peanuts (Virginia variety) obtained from Aperitivos Medina SL (Spain) were used in the study. Raw peanut seeds (1:10 w/v) were boiled in water, at 100 °C, for 60 min. Roasted peanuts seeds were autoclaved using a tabletop autoclave (CertoClav Multicontrol IPX4, Traun, Austria) at 121 °C (1.18 atm) for 15 and 30 min and at 138 °C (2.56 atm) for 15 and 30 min.

Raw and thermally processed peanut seeds were milled to pass through a 1 mm sieve (Tecator, Cylotec 1093, Höganäs, Sweden) and the resulting meal was defatted with *n*-hexane (34 ml/g of flour) for 4 h, shaken, and air-dried after filtration of the *n*-hexane. Defatted flour was extracted twice in a solution of 50 mM Tris–HCl, pH 8.0, plus 500 mM NaCl at a 1:10 w/v ratio for 1 h, at 4 °C, by stirring. After centrifugation (27000g, for 20 min, at 4 °C) the supernatants were dialyzed against H₂O (cut-off point, 3.5 kDa), for 48 h at 4 °C and freeze-dried. The protein content of each sample was measured according to the Bradford dye-binding assay (Bio-Rad, Hercules, CA) using BSA (Sigma, St. Louis, MO) as a standard.

2.3. Immunodetection assays

2.3.1. Protein electrophoresis and IgE immunoblot experiments

SDS–PAGE was performed according to Laemmli (1970). Samples (10 µg per well) were mixed with Laemmli sample buffer (Bio-Rad) and 2-mercaptoethanol (Bio-Rad) heated at 90 °C for 10 min, electrophoresed in 4–20% Tris–HCl linear gradient precast gel (Bio-Rad). Proteins were visualized with Coomassie brilliant blue R250 staining. Western blotting was performed by electrophoretic transfer to polyvinylidene difluoride (PVDF) membranes at 250 mA for 100 min, at room temperature, essentially according to the method of Towbin, Staehelin, and Gordon (1979). After blocking with 5% BSA (w/v) in PBS, membranes were incubated overnight with the serum pool from patients with clinical allergy to peanut (Nos. 5, 8, 9 and 11, 1:5 dilution) or individual sera (1:10 dilution) washed, and then treated with mouse anti-human IgE mAb HE-2 ascitic fluid (1:3000 dilution for 2 h) (Sanchez-Madrid, Morago, Corbi, & Carreira, 1984). After washing, a rabbit anti-mouse IgG peroxidase-conjugated antibody (1:5000 dilution for 1 h; DAKO, Glostrup, Denmark) was added. Detection of IgE-binding components was achieved by means of enhanced chemiluminescence, according to the manufacturer's instructions (Amersham Biosciences, Little Chalfont, United Kingdom). A serum pool (1:10 dilution) from three patients with specific IgE to *Anisakis* spp. was tested as a negative control.

2.3.2. ELISA

Specific IgE binding was determined by means of indirect ELISA in 14 individual sera from patients with clinical allergy to peanut (Nos. 2, 4, 6, 7, 9, 10, 12, 15, 17) and sera from patients sensitized to peanut (Nos. 21, 22, 24, 32, 33). The selection of these specific patients was made attending serum availability. Polystyrene 96-well microtiter plates (Costar 3590, Corning) were coated with 100 µl of extract at 30 µg/ml in PBS and incubated at 4 °C overnight. Wells were washed with PBS and 0.5% Tween 20 (v/v) and blocked with PBS containing 3% nonfat milk (w/v) and 0.1% Tween 20. Plates were incubated with individual sera (1:2 dilution) and binding of IgE was detected by mouse anti-human IgE mAb HE-2 ascitic fluid (1:5000 dilution for 1 h) (Sanchez-Madrid et al., 1984) followed by goat anti-mouse IgG peroxidase-conjugated (1:2500 dilution for 1 h, Pierce Chemical Co, Rockford, Ill). The peroxidase reaction was developed with 50 µl of peroxidase substrate buffer (Dako). After 30 min, the reaction was stopped with 50 µl of 4 N H₂SO₄, and the optical density (OD) was measured at 492 nm. All the tests were performed in triplicate.

2.3.3. Anti Ara h 1, Ara h 2 and Ara h 3 immunoblots

For anti Ara h 1, Ara h 2 and Ara h 3 experiments, defatted flours were solubilized with extraction buffer (50 mM Tris, 500 mM NaCl), followed by sonication and centrifugation at 5500g, for 15 min. The supernatant was removed and saved (soluble fraction). The pelleted fractions, after centrifugation (insoluble fraction), were solubilized by boiling for 5 min in standard electrophoresis sample buffer, containing 2% sodium dodecyl sulfate (SDS) and reducing agent (Invitrogen). The samples in SDS-sample buffer were centrifuged at 5500g for 15 min. The supernatants were removed, aliquoted and stored at –20 °C.

The proteins in the soluble and insoluble fractions were electrophoresed and transferred to PVDF membranes. Blocking was carried out for 1 h, at room temperature, in 5% blotto. Chicken anti-raw Ara h 1 (1:10000), chicken anti-raw Ara h 2 (1:8000) and chicken anti-raw Ara h 3 (1:5000) (custom synthesized by Sigma Immunosys, The Woodlands, TX) were diluted in 5% blotto and incubated with the PVDF membrane for 1 h, at room temperature. These antibodies have been shown to recognize both raw and thermally processed forms of these proteins (Schmitt, Nesbit, Hurlburt, Cheng, & Maleki, 2010). The HRP-labeled-anti-chicken IgY (1:100,000) (Sigma Immunosys, The Woodlands, TX), was diluted in 2% blotto and incubated with membranes for 30 min at room temperature. Detection was achieved as described above.

2.4. Digestion reactions with trypsin

Roasted peanut and autoclaved roasted peanut were selected for digestion experiments with trypsin. Soluble fractions were incubated in the presence of 0.0168 µM trypsin in a solution of 50 mM Tris, 1 mM EDTA at pH 8.4, at 37 °C. Aliquots were taken for SDS–PAGE analysis at 1, 3, 5, 10, 30 min, 1 h, 2 h, and overnight (~15 h). The gels were stained for 1 h using GelCode Blue Stain Reagent according to manufacturer's instructions (Pierce, Rockford, IL).

2.5. Circular dichroism (CD) spectroscopy

Far UV (185–250 nm) circular dichroism spectra of roasted peanut and autoclaved roasted peanut (1.18 atm and 2.56 atm, 15 and 30 min) were obtained. Samples were desalted through use of disposable gel filtration columns (Bio-Rad, Hercules, CA) into Milli-Q water and immediately used in CD measurements. Protein concentration was 0.1 mg/ml and spectra were obtained at room temperature with a JASCO 815 spectropolarimeter equipped with a Peltier temperature control system (Japan Spectroscopic Co. Ltd., Tokyo,

Japan). Average of two measurements was obtained using Jasco CD Manager Software. Data was analyzed with CDPPro.

2.6. Skin prick tests

Skin prick tests were performed through a standard technique with sterile needles (Alk-Abelló, Hørsholm, Denmark). Protein extracts of raw, boiled, fried, roasted and autoclaved roasted peanut (2.56 atm, 30 min) (10 mg/ml protein in PBS); a positive control (histamine dihydrochloride, 10 mg/ml); and a negative control (PBS) were applied on the volar side of the forearm in duplicate. The mean diameters of the wheal were measured after 15 min. To be considered positive, the wheal had to be at least 3 mm greater than that elicited by the negative control.

3. Results

3.1. Electrophoretic characterization

The SDS-PAGE patterns of the protein extracts of raw, boiled, fried, roasted and autoclaved roasted peanut seeds are presented in Fig. 1A1. Similar patterns were observed in raw, boiled, fried and roasted peanut extracts. Roasted peanut after autoclaving (1.18 and 2.56 atm) showed less stained bands but an increase of low molecular weight smear. Western blotting was performed using a serum pool from four patients with actual peanut allergy (Nos. 5, 8, 9 and 11) (Fig. 1A2). Similar allergenic proteins were present in raw, fried and roasted peanut extracts with the exception of a high molecular weight protein (121 kDa) that is significantly reduced in fried peanut extract (Fig. 1A2). The IgE-binding pattern of boiled peanut extract showed differences compared to

raw, roasted and fried peanut extract: the 65 kDa band (Ara h 1) and Ara h 2 (17–18 kDa) were less visible in this extract. After autoclaving of roasted peanut, a reduction in the total IgE-reactive bands was seen (Fig. 1A2). Bands of 121, 65, 37 kDa and two low molecular weight proteins (17, 14 kDa) were not bound by IgE in any autoclaved treatment (1.18 or 2.56 atm). Two immunoreactive bands of 26 and 33 kDa were still detected at high pressure (2.56 atm) applied for 15 min, however under the same pressure conditions applied during 30 min, none of them were recognized.

Western blotting using a serum pool from three patients with specific IgE to *Anisakis* spp. (negative control; Fig. 1A3) did not show any reactive bands.

IgE antibody reactivity to roasted peanut extract and autoclaved roasted peanut extract under extreme conditions (2.56 atm, 138 °C, 30 min) was further screened using fifty-four individual sera from patients sensitized to peanut. Western blotting of roasted (B1) and autoclaved roasted peanut extracts (B2) are shown in Fig. 1B. A complex pattern of bands from 13 to 121 kDa was detected in roasted peanut, with proteins of 65, 26, 22, 20 and 17 kDa recognized by 90%, 70%, 70%, 67.1% and 60% respectively. Twelve out of 54 (22%) sera recognized some proteins in autoclaved roasted peanut at 2.56 atm for 30 min.

3.2. ELISA

ELISA assay using roasted peanut and autoclaved roasted peanut (2.56 atm, 138 °C, 30 min) as a solid phase led to determine the specific IgE levels in the individual sera from fourteen peanut-sensitized patients. The percentage of the decrease in antigenic activity was calculated with the formula: $(1 - A_H/A_N) 100$ where A_H is the absorbance value obtained from autoclaved roasted peanut

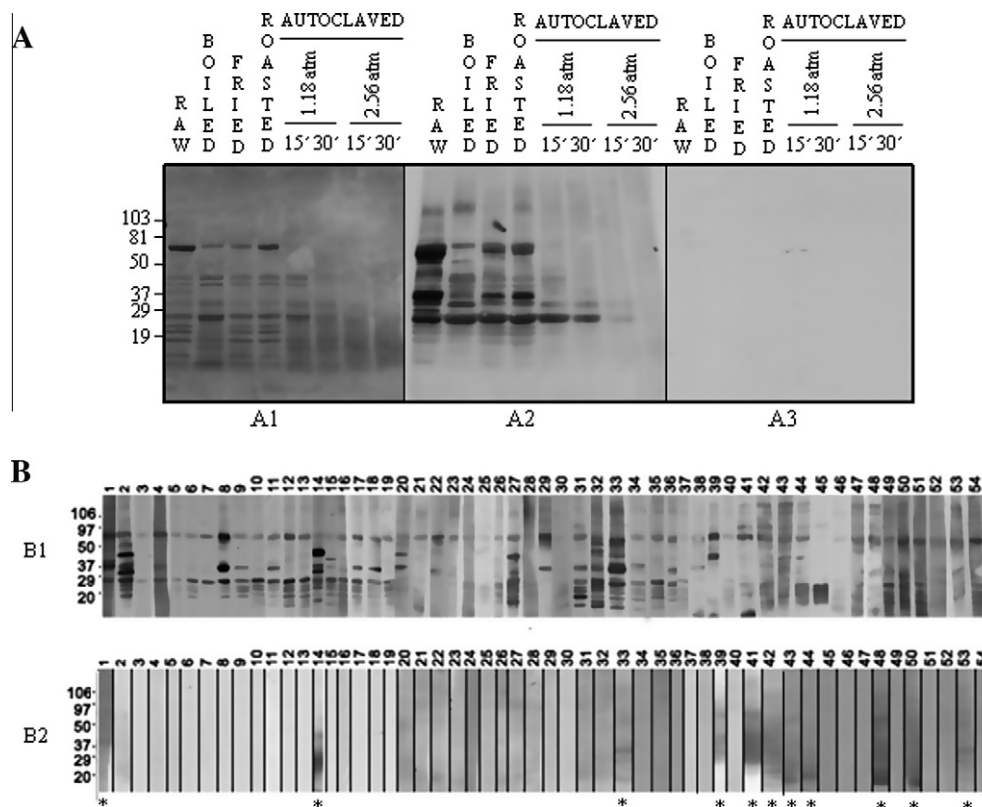


Fig. 1. A. SDS-PAGE (A1) and western blot (A2 and A3) of raw, boiled, fried, roasted peanut and autoclaved roasted peanut. Western blot A2 was carried out using a serum pool from 4 patients with actual peanut allergy (Nos. 5, 8, 9 and 11). Western blot A3 was carried out using a serum pool from 3 patients with allergy to *Anisakis* spp. 1B. Western blot of roasted peanut (B1) and autoclaved roasted peanut at 2.56 atm, 30 min (B2) incubated with sera from patients with actual peanut allergy (lanes 1–19) and sera from patients sensitized to peanut (20–54). Sera recognizing some proteins in autoclaved roasted peanut at 2.56 atmospheres for 30 min are marked with an asterisk.

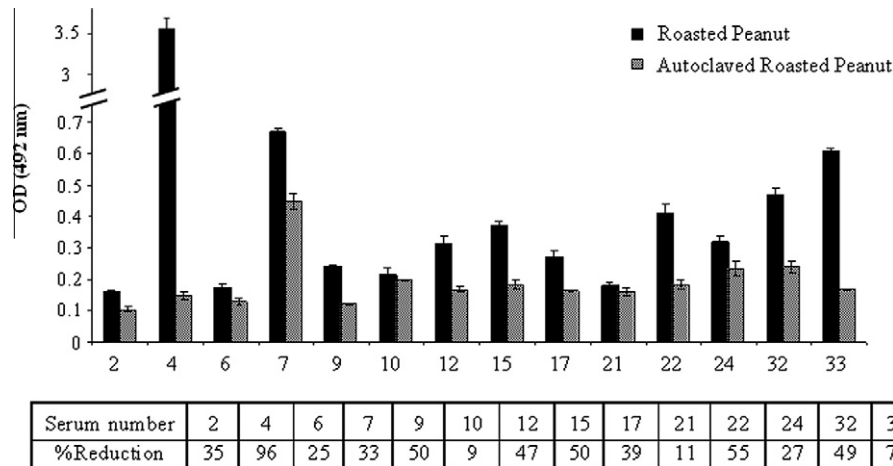


Fig. 2. ELISA of roasted peanut and autoclaved roasted peanut at 2.56 atm, 30 min, incubated with sera from patients with actual peanut allergy (Nos. 2, 4, 6, 7, 9, 10, 12, 15, 17) and sera from patients sensitized to peanut (Nos. 21, 22, 24, 32, 33). Percentages of decrease in antigenic activity are shown in the table annexed to Fig. 2.

samples and A_N is the absorbance value of the roasted peanut protein extract (Cabanillas et al., 2010).

The results are summarized in Fig. 2. Roasted peanut IgE reactivity was reduced by autoclaving at 2.56 atm, 30 min treatment in all sera tested. The treatment led to a minimum antigenicity reduction of 9% (in serum from patient 10) and a maximum antigenicity reduction of 96% (in serum from patient 4).

3.3. Ara h 1, Ara h 2 and Ara h 3 following autoclave processing

Solubility of proteins from roasted and autoclaved roasted peanut (1.18 and 2.56 atm, 15 and 30 min) was evaluated by SDS-PAGE extracting proteins into solution by buffer (soluble portion) and the pelleted portion of each sample, brought down by centrifugation following extraction of soluble material with buffer (insoluble portion) (Fig. 3A).

Soluble fractions showed a reduction in the overall level of intact proteins following autoclave treatments compared with insoluble fractions. Specific anti Ara h 1, anti Ara h 2 and anti Ara h 3 antibodies used to identify Ara h 1, Ara h 2 and Ara h 3 molecules in soluble and insoluble fractions of roasted and autoclaved roasted peanut (Fig. 3B) confirmed the results observed in SDS-PAGE. A decrease in the relative Ara h 1, Ara h 2 and Ara h 3 levels could be observed in autoclaved roasted peanut samples with increased pressure and time, in the soluble fraction compared to the insoluble fraction. Nevertheless, these results are more evident in Ara h 1 than Ara h 2 and Ara h 3 immunoblots. The relative level

of Ara h 1 increased in the insoluble fraction of autoclaved roasted peanut compared to soluble fraction, and high-molecular-weight-aggregates or oligomers could be observed in the insoluble fraction. However, in spite of these facts, there was a marked decrease in recognition of Ara h 1, Ara h 2 and Ara h 3 in autoclaved roasted peanut at 2.56 atm, 30 min even in the insoluble fractions.

3.4. Susceptibility of autoclaved roasted peanut extract to trypsin

To evaluate digestibility, roasted peanut and autoclaved roasted peanut (1.18 atm, 15 and 30 min) were subjected to trypsin treatment (Fig. 4). SDS-PAGE separation followed by immunodetection with a serum pool from three patients with clinical allergy to peanut (Nos. 7, 12, 15) indicated that autoclaved roasted peanut, compared to roasted peanut, was more extensively digested by trypsin within 10 min of treatment, losing most of its capacity to bind IgE from peanut allergic patients (Fig. 4B).

3.5. CD analysis of the structural alterations

The secondary structure of roasted peanut and autoclaved roasted peanut (1.18 atm and 2.56 atm, 15 and 30 min) was analyzed by CD spectroscopy and the far-UV spectra are shown in Fig. 5. The percentages of α -helix, β -sheet and random coil and/or loops are shown in the table annexed to Fig. 5. The CD spectrum of roasted peanut proteins rendered 50% α -helix, 10% β -sheet, and 40% random coil and/or loops. As can be seen in the case of auto-

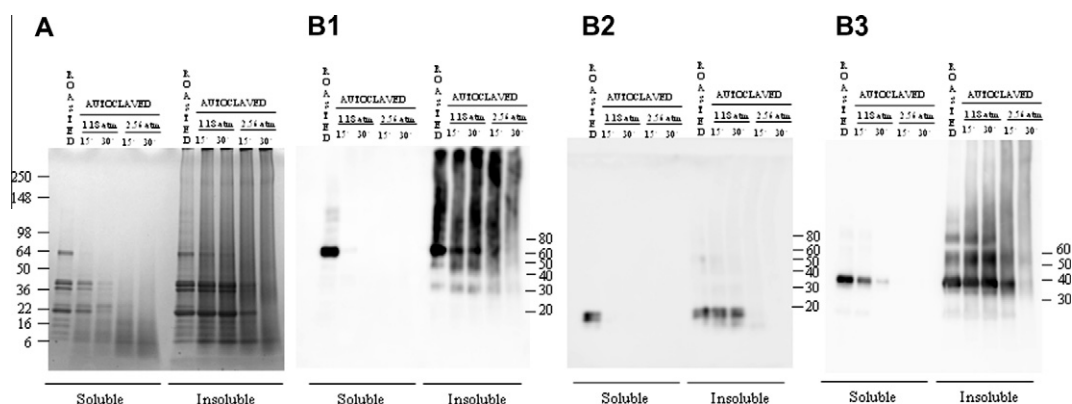


Fig. 3. SDS-PAGE (A) and anti-Ara h 1 (B1), anti-Ara h 2 (B2) and anti-Ara h 3 (B3) western blot of soluble and insoluble fractions of roasted and autoclaved roasted peanut.

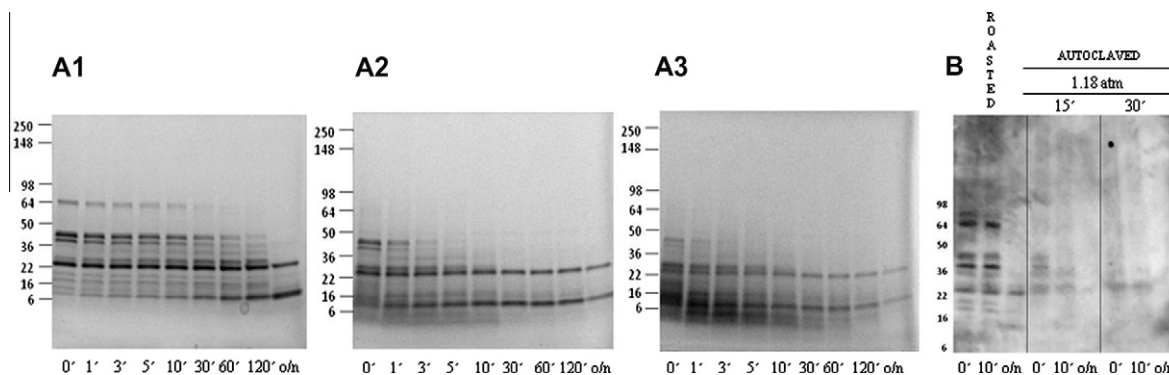
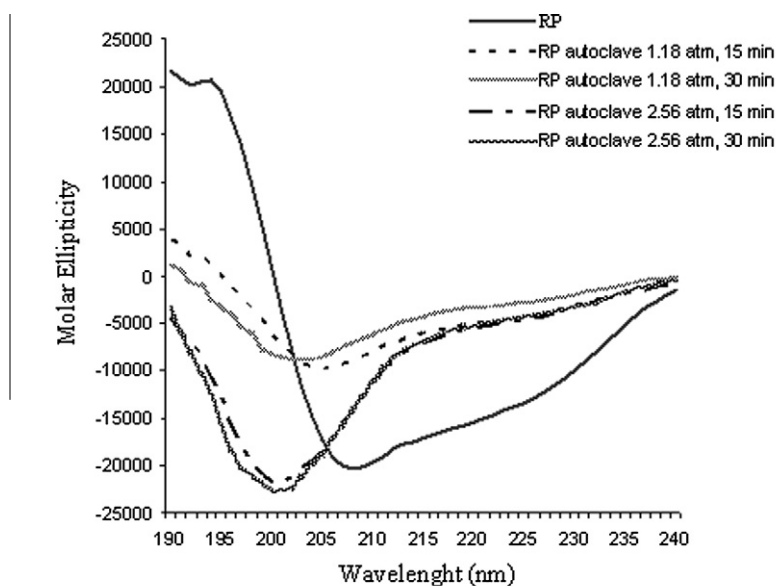


Fig. 4. A. SDS-PAGE analysis of the digestion of roasted peanut (A1), autoclaved roasted peanut 1.18 atm, 15 min (A2) and autoclaved roasted peanut 1.18 atm, 30 min (A3) by trypsin at 0, 1, 3, 5, 10, 30, 60, 120 min and overnight (~15 h). B. Western blot of selected digestion times (0, 10 min and overnight) of roasted peanut and autoclaved roasted peanut using a serum pool from 3 patients with clinical allergy to peanut (Nos. 7, 12 and 15).



	α -helix	β -strand	Random coil and/or loops
RP	50	10	40
RP autoclave 1.18 atm, 15 min	16	24	60
RP autoclave 1.18 atm, 30 min	7	26	67
RP autoclave 2.56 atm, 15 min	15	7	78
RP autoclave 2.56 atm, 30 min	11	9	80

Fig. 5. CD-spectrum of roasted peanut (RP) and autoclaved roasted peanut at 1.18 atm and 2.56 atmospheres (15 and 30 min). Percentages of α -helix, β -strand and random coil and/or loops are shown in the table annexed to Fig. 5.

claved roasted peanut, most of the α -helical structure was lost (11% remaining in autoclaved roasted peanut at 2.56 atm, 30 min), random coil and/or loops increased (80% in autoclaved roasted peanut at 2.56 atm, 30 min) and β -sheets remain.

3.6. Skin prick tests

Table 1 shows the results of skin testing with raw and processed peanut extract samples performed on seven patients with clinical allergy to peanut. All patients had skin reactivity to raw, fried and roasted peanut extract, although six out of seven reactions were positive with roasted peanut. Boiled peanut extract elicited positive reactions in one patient. None of the patients reacted to autoclaved roasted peanut extract. Healthy subjects did not have skin reactivity to any protein extract.

4. Discussion

In this study the effect of different food-processing conditions on peanut allergenicity was investigated. The results showed that IgE immunoreactivity of roasted peanut protein extract decreased significantly at extreme conditions of autoclaving (2.56 atm, 30 min), as shown by *in vitro* experiments of western blot, ELISA and *in vivo* experiments of skin prick tests. These results can be explained by circular dichroism experiments: most of the α -helical structure was lost after autoclave treatments. It is known that many of the IgE binding epitopes in the major allergens of peanuts (i.e. Ara h 1, Ara h 2 and Ara h 3) are located on the α -helical regions of these proteins (Barre, Jacquet, Sordet, Culerrier, & Rougé, 2007; Mueller et al., 2011; Shin et al., 1998). However, although the decrease of IgE-binding proteins is notable, *in vitro* experi-

Table 1

Results of skin prick testing with raw and thermal processed peanuts in patients with clinical allergy to peanut.

SPT (mm) peanut						Immunological and clinical features	
Patient	Raw	Boiled	Fried	Roasted	Autoclaved roasted	CAP-FEIA (kU/L)	Diagnostic challenge
A	4.2	0	3.5	3.5	0	1.26	DBPCFC
B	7.5	0	6.2	5	0	0.2	DBPCFC
C	4.5	0	4.2	2.2	0	0.95	DBPCFC
D	7	3	5.6	5.6	0	6.7	DBPCFC
E	6.5	1.8	5.5	4	0	3.5	DBPCFC
F	7.2	1.5	6	5.5	0	0.8	— ^a
G	6.2	3.7	4.8	5.5	0	4	— ^a

CAP-FEIA, Capsulated hydrolytic carrier polymer-fluoro-enzyme immunoassay; DBPCFC, double-blind, placebo-controlled food challenge; SPT, skin prick testing.

^a Not challenged because of a convincing history of severe anaphylaxis with peanut.

ments showed that 22% of sera recognized some proteins in autoclaved roasted peanut at 2.56 atm, for 30 min, in western blot. ELISA results showed that autoclaved roasted peanut (2.56 atm, 30 min) elicited a decrease of IgE reactivity between 9% and 96%.

These findings suggest that autoclaving produces an important decrease in IgE-binding properties of roasted peanut due to changes in the structure of the proteins. Similar results have been previously reported in other legumes: Malley, Baecher, Mackler, and Perlman (1975) found that autoclaving green pea at 120 °C for 15 min reduced its allergenicity. Also, IgE-binding capacity of lupine allergens was reduced by autoclaving at 2.6 atm, for 30 min, or by DIC (instantaneous controlled pressure drop) at 6 bar (5.9 atm) for 3 min, whereas it was only slightly affected by boiling, microwave, and extrusion-cooking (Alvarez-Alvarez et al., 2005; Guillamón et al., 2008). However, it has been demonstrated that the allergenicity of the almond major protein (amandin) and a peach protein in the nectar (Pru p 1) is maintained after autoclaving at 121 °C, up to 30 min (Brenna et al., 2000; Venkatachalam, Teuber, Roux, & Sathe, 2002).

Autoclaved roasted peanut, compared to roasted peanut, was more extensively digested by trypsin within 10 min of treatment, losing most of its capacity to bind IgE from peanut allergic patients. It has been demonstrated that heat treatments can increase the susceptibility to enzymatic digestion of some allergens (Morisawa et al., 2009). In the present study, circular dichroism experiments showed unfolding of proteins in autoclave treated samples, which makes them more susceptible to digestion. Morisawa et al. (2009) found that B cell epitopes of β -lactoglobulin were impaired by heat treatment, increasing susceptibility to digestion and facilitating the enzymatic cleavage of protein sequences that disrupts linear B cell epitopes.

The degree of processing can dramatically affect the results of digestibility, solubility and other parameters (Maleki, 2004). During processing, proteins can form oligomers, become denatured, degraded, aggregated, cross-linked, fragmented and re-assembled and these changes most often cause a reduction in solubility (Maleki, 2004). In this way, processing can alter the overall IgE binding profiles of a particular extract (Schmitt et al., 2010). It can be hypothesized that the soluble portion of autoclaved roasted peanut samples assessed in this study contains less allergenic proteins or products due to a reduction in the solubility caused by autoclave treatment. For this reason, protein profiles, including peanut allergens: Ara h 1, Ara h 2 and Ara h 3, were assessed in soluble and insoluble fraction of roasted and autoclaved roasted peanut. The results showed that the total level of intact protein decreases with increased pressure and time in both, soluble and insoluble fractions, although soluble fractions showed a higher reduction of proteins than insoluble fractions. Ara h 1, Ara h 2 and Ara h 3 allergens were recognized more extensively by specific antibodies in insoluble fraction than in soluble fraction. Moreover, Ara h 1 was present in higher molecular weight aggregates or oligomers in the insoluble fraction. It has been demonstrated that

heating of purified Ara h 1 leads to a more structured secondary conformation of the protein, with an increased content of extended β -sheet structures leading to the formation of large protein complexes or aggregates (Koppelman, Bruijnzeel-Koomen, Hessing, & de Jongh, 1999). However, in the present study, a marked decrease of Ara h 1, Ara h 2 and Ara h 3 allergens was detected at harsh conditions of autoclaving (2.56 atm, 30 min) in both, soluble and insoluble, fractions.

In summary, although further studies are needed to assess the clinical relevance of our findings, we can conclude that autoclaving at 2.56 atm for 30 min produces a significant decrease of IgE-binding capacity of peanut allergens due to changes in their secondary structure. This treatment could be a technique to improve food safety.

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